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WATER STRESS MANAGEMENT USING HYDROGELS AND THEIR IMPACT ON PLANT BIOCHEMICAL COMPOSITION OF *LACTUCA SATIVA*

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Abstract

In the recent decades, hydrogels have proven great potential for agricultural usage regarding water management and controlled release of stimulants and further improving the food production. The aim of this study is to analyse the effects of 4 different hydrogel compositions in 2 forms of administration (beads and granules) on water management and the impact on the biochemical composition of lettuce (Lactuca sativa). In this regard, two different conditions were assured, namely induced drought stress and regularly irrigated conditions. The lettuce plants were periodically analysed to determine the ascorbic acid content, polyphenolic content and antioxidant activity. The results showed slightly higher content in ascorbic acid for the regularly irrigated plants. Contrary, the antioxidant activity and total polyphenolic content showed an increase in plants subjected to drought stress. Between the two forms of hydrogel administration, the granular form presented better properties in alleviating the water stress induced by the extensive period of drought.

***Key words**: biochemical parameters, hydrogels, water stress.*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most consumed and farmed leafy vegetable in the world, with output increasing year after year. In 2018, the total harvested area was anticipated to be more than 1.27 million hectares, with a total yield of roughly 27.3 million tons (Medina-Lozano et al., 2021).

Due to water shortage, irrigation water conservation is becoming increasingly critical in lettuce output to achieve optimal yield. Furthermore, due to rising human demand, water supplies for agriculture is sometimes insufficient. Given the increased focus on the efficient use of precious water resources, it is critical to enhance water usage efficiency (Chen et al., 2019). Drought has a negative influence on plant development, growth, and fertility. Long-term drought stress and increased stress severity result in further acclimation processes. These responses include osmotic adjustment, reduced shoot-root ratio, cell wall changes, metabolic reprogramming, and antioxidant system activation (Laxa et al., 2019).

Hydrogels have developed as an efficient technology for increasing soil water-holding capacity and conserving moisture, particularly in dry and semi-arid environments. These crosslinked polymers, known as hydrophilic gels, absorb water without disintegrating. They boost soil water availability for plants, reduce evaporation and percolation waste, and improve crop development and yield characteristics (El Bergui et al., 2023). Hydrogels function as "miniature water reservoirs" around plant roots. They can collect natural and provided water from 400 to 1500 times their own weight and slowly release it under water-stress circumstances via the root capillary suction mechanism (Patra et al., 2022).

Lettuce is perceived as healthy food by consumers. The nutritional qualities are mostly ascribed to the crop's provision of antioxidant chemicals, such as vitamin C, polyphenols, and vitamin E (Medina-Lozano et al., 2020). It is a rich source of bioactive chemicals with a wide

range of biological activity, including antioxidant, anti-inflammatory, anticancer, antibacterial, cholesterol-lowering, and anti-diabetic properties, as well as an excellent source of fiber, iron, and folate (Mohamed et al., 2021).
One of the most important indications of the nutritional quality of fruits and vegetables is vitamin content, particularly vitamin C. Its beneficial properties are related to its role in various processes in the human body, such as collagen formation, cholesterol reduction, inorganic iron absorption, and, most importantly, immune system enhancement due to its antioxidant activity (Medina-Lozano et al., 2021). Vitamin C is a required vitamin for humans, unlike many other animals, because we are unable to synthesize it due to mutations in the gene coding for the last step enzyme in the biosynthesis pathway. It is necessary for regular cell metabolism and also plays a vital role in immunological responses, owing to its antioxidant action (Medina-Lozano et al., 2020).
The presence of phenolic chemicals in lettuce is regarded to be the fundamental cause for its high antioxidant activity. They are formed in response to environmental stress and other stressors such as UV, radiation, injuries, and processing procedures including postharvest treatment (Materska et al., 2018). Recent studies (Zhang et al., 2023; Hernández-Adasme et al., 2023; Utami & Damayanti, 2023) revealed antioxidant activity in lettuce leaves. Furthermore, lettuce's preventive benefit can be attributed in part to its high antioxidant content, which includes antioxidative enzymes, flavonoid, ascorbate, carotenoid, and tocopherol (Nikzad & Parastar, 2021). The amount of phenolic substances (most notably flavonols, caffeic acids and their derivatives, carotenoids, and vitamins C and E) and antioxidant activity are also noted in lettuce and they are heavily impacted by genetic variables, environmental circumstances, and production systems, among other things (Negrao et al., 2021).
Therefore, this study aimed to analyze the bioactive compounds such as total phenolic content, vitamin C and the antioxidant activity of *Lactuca sativa* under drought stress conditions, using hydrogels based on different concentrations of montmorillonite.

MATERIALS AND METHODS

1. Polymeric Material

The hydrogels used in this experiment were obtained from The National Institute for Laser, Plasma and Radiation Physics, Măgurele, using the electron beam radiation method and potassium persulfate as activator. Four different compositions of hydrogels based on acrylic acid, polyethylene oxide, sodium alginate and montmorillonite were tested. In this experiment two methods of administration of the hydrogels were tested, namely granular form (VG) and beads (VB) (Figure 1, Table 1). Each type of hydrogel was applied in 10 repetitions (5 for drought conditions and 5 for irrigated conditions) for each composition, the weight of 1 bead being of 0.2 g, weight that was kept also in the case of granules. The hydrogels were buried in 50 g soil and 100 ml water was added for each sample to assure the swelling of the polymeric material.

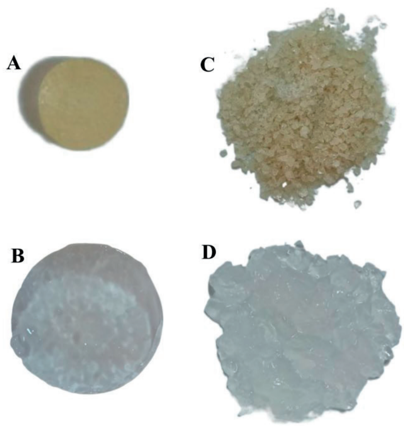

Figure 1. Hydrogel sample:
A. Hydrogel bead; B. Hydrogel bead swollen in water; C. Hydrogel granules; D. Hydrogel granules swollen in water

Table 1. Sample codification used in this study

Code	Type of hydrogel		Montmorillonite concentration (%)
	Bead	Granular	
V1	V1B	V1G	0
V2	V2B	V2G	0.25
V3	V3B	V3G	0.5
V4	V4B	V4G	1
M	Control sample with no hydrogel and drought conditions		

2. Plant Materials and Growing Conditions
The lettuce (*Lactuca sativa*) seedlings placed in alveolar tray, were acquired from SCDL Buzău and used in the experimental tests as follows: the seedlings were successively transplanted in cups, pots and after a period of 35 days they were planted in a greenhouse (Figure 2) where 2 different conditions were applied, one simulating the drought stress conditions (without irrigation) and the other with regularly irrigated plants.

A B C D

Fig. 2 Lettuce sample (*Lactuca sativa*) on different stages of growth:
A. Lettuce seedling; B. Lettuce transplanted in cups; C. Lettuce sample after 21 days (in pot); D. Lettuce sample after 25 days (in greenhouse)

3. Biochemical analysis
Biochemical determinations were further conducted, in order to determine the content in vitamin C, total phenolics and antioxidant activity.
Vitamin C. For the total vitamin C content determination, 10 g of fresh lettuce leaves were grounded and mixed with 20 ml of 2% oxalic acid. The obtained mixture was brought to 100 ml and let to extract for 15 minutes. Further, 2 ml of the filtered extract was mixed with 1 ml of 2% oxalic acid, 5 ml buffer solution, 2 ml of indophenol (2,6-Dichlorophenol Indophenol) and 20 ml xylene. The obtained solution was then centrifuged for 20 min at 4°C and 9000 rpm. Sample absorbance was measured at 500 nm and the results were expressed as mg/100g dry weight (DW).
Total phenolic content. The Folin–Ciocalteu method was used to determine total phenolic content. Briefly, 5 g of fresh chopped leaves were extracted in 25 ml ethanol 75% for a period of 48h in the dark. Further, 20 µl of filtered extract was mixed with 1.58 ml distilled water, 100 µl Folin-Ciocalteu reagent and 300 µl of 20% sodium carbonate. The obtained samples were incubated at room temperature for 2h in the dark, and then, the absorbance was measured at 765 nm. The results were expressed as mg GAE (gallic acid equivalents) /100g dry weight (DW).
Antioxidant activity. For antioxidant activity determination, the DPPH method was used. Therefore, 5 g of fresh chopped leaves were extracted in 25 ml ethanol 75% for a period of 48h in the dark. Then, 0.05 ml of filtered sample was mixed with 1.950 ml DPPH solution. After incubation at room temperature for 30 min in the dark, the absorbance of the samples was measured at 515 nm and the results were expressed as mg QE (Quercetin Equivalents)/100 g dry weight (DW).

RESULTS AND DISCUSSIONS

The biochemical analyses were performed initially and after 3, 20 and 40 days from the initiation of the simulated drought process.

Vitamin C content
The vitamin C content during the drought induced stress after 3 days showed that the samples containing higher concentrations of montmorillonite are proven to be more beneficial as seen in Figures 3 and 4. After 20 days of drought the progressive decrease of vitamin C content can be noticed, the samples that contained no montmorillonite having the lowest amount of ascorbic acid concentration, similar to the Control sample. After 40 days under drought conditions, the vitamin C content decreased considerably for each sample showing a noticeable difference between the Control and the plants grown in the presence of hydrogels (either bead or granular form hydrogels). Between the 2 forms of administration, the bead form showed better results for the vitamin C content (Figures 5 and 6).

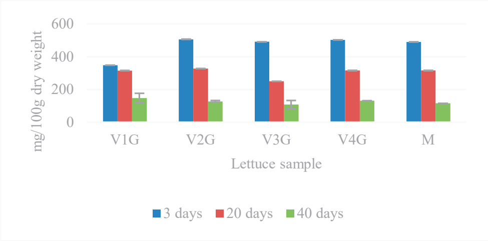

Figure 3. The evolution of vitamin C accumulation under induced drought for the granular hydrogels

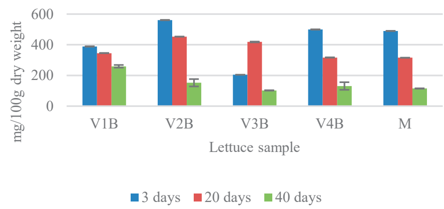

Figure 4. The evolution of vitamin C accumulation under induced drought for the bead hydrogels

The ascorbic acid content during the drought conditions showed higher values for both forms of administration compared to the usual content range of vitamin C in *Lactuca sativa*. In a review conducted by Kim et al. (2016) on different lettuce types, the range of ascorbic acid oscillated between 8.40 and 390 mg/100 g DW. The result of our study showed that vitamin C values remained in the range, even after the 40 days of induced drought stress, the values oscillating between 107 and 259 mg/ 100 g DW.

After 40 days, the vitamin C content was slightly higher for the plants under irrigated conditions as showed in Figure 5. Compared to the Control, most samples showed higher content of ascorbic acid for both forms of hydrogel administration and irrigating conditions.

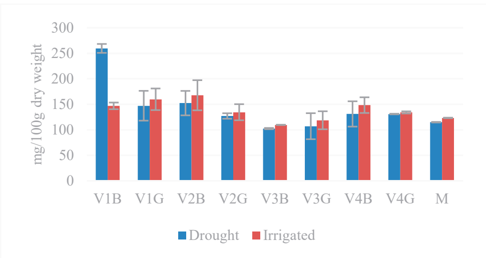

Figure 5. Vitamin C content after 40 days of induced drought stress and regular irrigation

Antioxidant activity

The antioxidant activity under drought conditions as showed in Figures 6 and 7, proved to be higher as the stress induced by the drought increases over time. Therefore, the longer the period of induced drought stress, the higher the antioxidant activity, as reported also by other authors (Liava et al., 2023; Pan et al., 2023).

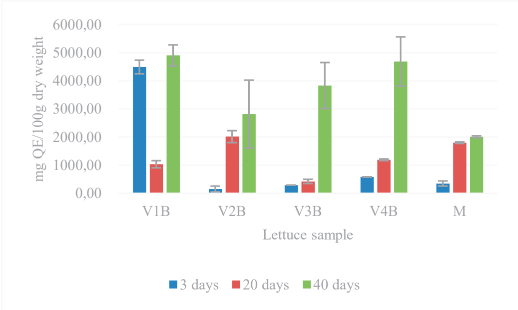

Figure 6. Evolution of the antioxidant activity under induced drought stress for bead hydrogels

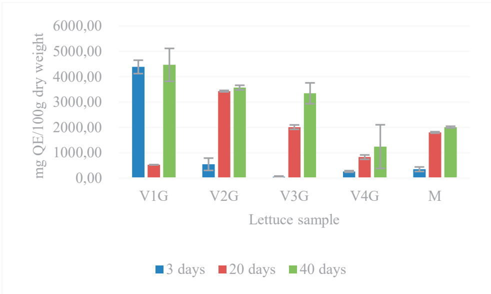

Figure 7. Evolution of the antioxidant activity under induced drought stress for granular hydrogels

The plants that were exposed to drought conditions presented significantly increased antioxidant activity for both forms of administration of hydrogels compared to the Control sample, most likely as a metabolic response to water stress, as it is also described in literature by various authors for different plant species (Talbi et al., 2020).

Samples in the presence of hydrogels with lower concentrations of montmorillonite (V2-V3) on either form of administration proved to have considerable effect on reducing the stress induced by the severe drought conditions, slightly similar to the Control plants (Figure 8).

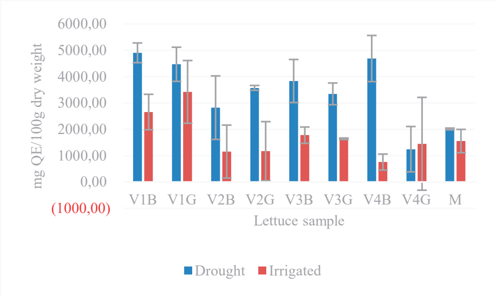

Figure 8. The antioxidant activity after 40 days of induced drought stress and regular irrigation

Total polyphenolic content

The total polyphenolic content showed similar results as the ones obtained for antioxidant activity under the drought induced stress. Higher values were observed as the drought period increased (Figures 9 and 10).

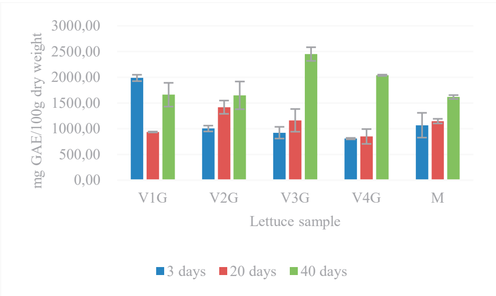

Figure 9. Evolution of the total polyphenol content under induced drought stress for granular hydrogels

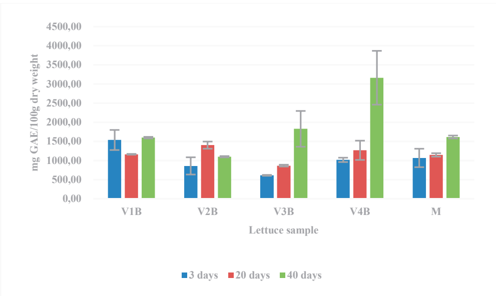

Figure 10. Evolution of the total polyphenol content under induced drought stress for bead hydrogels

In a study conducted by Liu et al. (2007) on different types of *Lactuca sativa*, they obtained the total phenolic content in a range between 1840-5640 mg GAE/100 g dry weight. Results of our study showed lower content in total phenolics, the values oscillating between 613 (V3B) and 3164 (V4B) mg GAE/100 g DW, which increased over time during the drought induced stress.

After 40 days, the total polyphenolic content proved to be higher for the plants that were exposed to the drought conditions, similarly to the results regarding the antioxidant activity. Samples containing hydrogels with higher concentrations of montmorillonite (V3-V4) presented better results regarding the total polyphenols accumulation during both drought stress as well as under irrigated conditions (Figure 11).

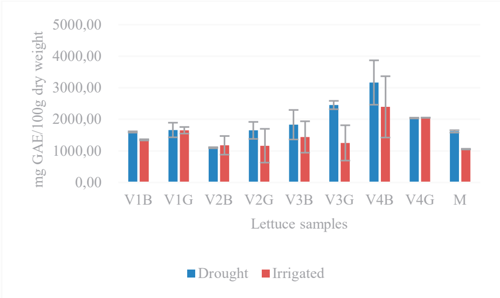

Figure 11. The polyphenols content after 40 days of induced drought stress and regular watering conditions

Therefore, analysing the obtained results, a selection of the form of administration of the hydrogel (bead or granules) can be made, based on the correlation that exists between the water stress and the antioxidant activity of the plant, namely the longer the period of exposure to water stress, the higher the antioxidant activity. Thus, according to the results of this study (Figure 12), we can conclude that for all the variants of tested hydrogels and both methods of administration (V1, V2, V3, V4), the granular form proved to alleviate the water stress induced by the extensive period of drought, compared to the bead administration form of the same hydrogels and even compared to the Control for V4 samples.

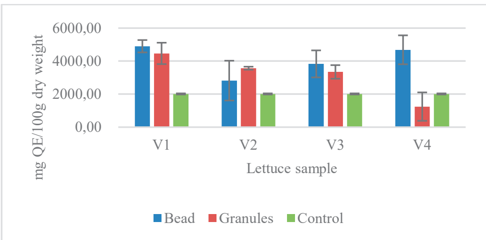

Figure 12. The antioxidant activity of Lactuca sativa after 40 days of drought stress

CONCLUSIONS

The results showed that the usage of hydrogels containing montmorillonite proved to be beneficial for water management regarding long periods of drought. Between the two forms of administration, the granular form of hydrogels was the best in alleviating the water stress induced by the extensive period with no irrigation compared to the bead administration form of the same hydrogels. The samples containing higher amount of montmorillonite proved to have considerable effect on reducing the stress induced by the severe drought conditions, slightly similar to the control plants while also presenting better results regarding the total polyphenols accumulation during both drought stress as well as under irrigated conditions. The ascorbic acid content was slightly higher for the regularly watered plants. However, the obtained results showed that the values of the studied parameters at the end of the testing period were not significantly different between the two cultures; therefore, the tested hydrogels present great potential to be used for water management in agriculture. Furthermore, higher concentrations of montmorillonite in the hydrogel composition showed increased tolerance to drought stress compared to no or lower concentrations as well as the Control plants.

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